Arabian Journal of Chemistry (2018) xxx, xxx–xxx

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

An efficient sample preparation method based on dispersive liquid–liquid microextraction associated with back extraction for trace determination of acidic pharmaceuticals

Mahnaz Ghambarian [a,*], Fateme Tajabadi [a], Yadollah Yamini [b], Mohammad Behbahani [c], Hamid Reza Sobhi [d], Ali Esrafili [e,f]

[a] *Iranian Research and Development Center for Chemical Industries, ACECR, Tehran, Iran*
[b] *Department of Chemistry, Faculty of Sciences, Tarbiat Modares University, Tehran, Iran*
[c] *Faculty of Engineering, Shohadaye Hoveizeh University of Technology, Dasht-e Azadegan, Susangerd, Iran*
[d] *Department of Chemistry, Payame Noor University, Tehran, Iran*
[e] *Research Center for Environmental Health Technology, Iran University of Medical Sciences, Iran*
[f] *Department of Environmental Health Engineering, School of Public Health, Iran University of Medical Sciences, Tehran, Iran*

Received 4 November 2017; accepted 23 February 2018

KEYWORDS

Dispersive liquid–liquid microextraction;
Back-extraction;
Non-steroidal anti-inflammatory drugs;
Gas chromatography;
High performance liquid chromatography

Abstract Reduction of matrix effect seems to be a great challenge for the development of a practical method in bioanalysis. In this regard, a simple and efficient DLLME procedure along with a back-extraction step (DLLME-BE) was developed for the preconcentration of four common non-steroidal anti-inflammatory drugs (NSAIDs) in various biological fluid samples. Briefly, the analytes of interest were initially transferred into the extraction solvent followed by the back-extraction into an immiscible basic methanol (as an acceptor phase) for further preconcentration and clean-up. The main purpose of the work is reducing the matrix effect and sensitive determination of target molecules in the complex matrices. Following on, the separation and determination of the analytes were carried out using GC–MS (in-port derivatization) and HPLC-DAD instrument. The influential parameters affecting the DLLME-BE method were evaluated in detail and the best extraction conditions were established. Under the optimum conditions, low method detection limits in the range of 0.1–1.0 and 0.1–6.0 μg L^{-1} were obtained for GC–MS and HPLC-DAD analysis,

* Corresponding author.
E-mail address: ghambarian.m@gmail.com (M. Ghambarian).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.arabjc.2018.02.010

Please cite this article in press as: Ghambarian, M. et al., An efficient sample preparation method based on dispersive liquid–liquid microextraction associated with back extraction for trace determination of acidic pharmaceuticals. Arabian Journal of Chemistry (2018), https://doi.org/10.1016/j.arabjc.2018.02.010

respectively. Additionally, fair intra-day precisions of 2.7–14.5 and 2.8–7.8% as well as inter-day precisions of 3.9–14.5 and 3.5–8.1% were achieved for the GC–MS and HPLC-DAD analysis, respectively. Finally, the method was successfully applied for the determination of four common NSAIDs in different biological fluid samples.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are classified as pharmaceuticals and personal care products (PPCPs) (Zhang et al., 2013) commonly prescribed for the treatment of a wide variety of pain-related diseases. This class is made up of salycilic acid, ibuprofen, ketoprofen, naproxen, fluribuprofen, diclofenac, phenoprofen and ketorolac. Although NSAIDs are regarded as low-risk, they may cause intensive toxic effects in cases of acute over-dosage or chronic abuse (Kim and Aga, 2007). Thus, their detection in biological fluid samples is of great importance.

Despite a huge progress recently made in the sample pretreatment procedures, sample pretreatment is still regarded as the crucial step in the modern analytical techniques. Generally, turning an analyte into a pre-purified, concentrated, which is compatible with the analytical instrument, is vital in the sample preparation. Many attempts have been made for the development of such efficient, low-cost, and miniaturized sample preparation methods. So far, various modes of solid- and liquid-phase microextraction (LPME) (Bazregar et al., 2016; Barfi et al., 2015; ParrillaVázquez et al., 2013; Toledo-Neira and Álvarez-Lueje, 2015) have been developed. LPME is an emerging technique, which is based on the low consumption of organic solvents, mainly used for the extraction of analytes from aqueous matrices.

Further on, Rezaee et al. reported a LPME method known as DLLME (Rezaee et al., 2006), which has gained tremendous attentions for its simplicity and ability to provide high extraction efficiencies within a short period of time. Nevertheless, DLLME as a one-step extraction technique, suffers from inadequate clean-up and low reproducibility in trace analysis associated with highly complicated matrices (Guo and Lee, 2013; Simão et al., 2016). To reduce the mentioned problems, the coupling of DLLME with back-extraction step based on the implementation of two immiscible organic solvents, as an improved kind of DLLME, was first introduced by Ghambarian et al. (2016).

To analysis of non-steroidal anti-inflammatory drugs a sample preparation step is necessary for reducing of matrix effect before analysis by instrumental analysis (Mzukisi Madikizela et al., 2018; Kamaruzaman et al., 2013; Sultan et al., 2005; Bazregar et al., 2016; Gouda et al., 2013).

In the present work, a DLLME-BE method prior to HPLC-DAD/GC–MS analysis was applied for the preconcentration of the selected NSAIDs (ibuprofen, naproxen, ketoprofen, and diclofenac). In fact, the back-extraction step improves sensitivity, reproducibility and clean-up to a great extent. The novelty of the work was back extraction step that provided two main advantages in comparison to previously DLLME methods as follows:

1- In fact, we did extract the biological samples using the DLLME stage alone and what we achieved was the organic extract phase (2-dedcane and TOPO) full of unwanted materials which overwhelmed the whole phase resulting in an enhancement of background signals and interferences within the matrix.
2- Additionally, the introduction of the above-mentioned extract phase (2-dedcane and TOPO) to GC and HPLC could hamper the whole analysis and does severe damages to the used chromatographic instruments.

In GC–MS case, since the studied NSAIDs (ibuprofen, naproxen, ketoprofen, and diclofenac) are classified as polar carboxylic acids, they should be identified and quantified in their corresponding silyl ester forms (Rodriguez et al., 2003; Yu et al., 2007; Rice and Mitra, 2007). The silyl derivatives were prepared using N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) acting as a silylating reagent. It is worth noting that the analytes were instantly derivatized inside the hot GC–MS injector port (in-port derivatization), which eliminates the lengthy reaction time needed in conventional in-vial silylation.

Considering the above-mentioned points, for these polar analytes the back-extraction step not only improved the reproducibility and clean-up but also minimized the matrix effect prior to both chromatographic analyses.

2. Materials and methods

2.1. Materials and samples

Naproxen (NAP), Ketoprofen (KET), ibuprofen (IBU), and diclofenac (DIC) (all with the purity of ≥98%) used in this study were supplied by Sigma–Aldrich (Steinheim, Germany). MSTFA (99.8% purity), potassium hydroxide, trioctylphosphine oxide (TOPO) and Trifluoroacetic acid (TFA) were also purchased from Sigma–Aldrich (Steinheim, Germany). Ultrapure water and HPLC-grade methanol were purchased from Samchun (Pyeongtaek, South Korea). Urine and plasma samples containing the drugs were delivered by two patients. All samples were collected in accordance with the ethical guidelines and permission of institutional review board (IRB), and the urine and plasma samples were stored at 4 °C and −20 °C, respectively. The drug-free plasma and urine samples were provided from the Hakim Medical Clinic (Tehran, Iran) and used for the plotting the calibration curves (matrix-matched calibration). The pH of the sample solutions were adjusted at 1.0 by a drop-wise addition of 1 mol L^{-1} HNO_3 solution. A stock solution (1000 mg L^{-1} in MeOH) of each drug was prepared and stored at 4 °C. The stock solutions were then mixed and used for the fortification of water, urine and plasma samples. Method optimization was

Please cite this article in press as: Ghambarian, M. et al., An efficient sample preparation method based on dispersive liquid–liquid microextraction associated with back extraction for trace determination of acidic pharmaceuticals. Arabian Journal of Chemistry (2018), https://doi.org/10.1016/j.arabjc.2018.02.010

performed using the ultrapure water fortified at the level of 100 μg L^{-1} with respect to each drug.

2.2. Instrumental

An Agilent HPLC instrument (Wilmington, USA) equipped with an Agilent G1315D diode array detector (DAD), a 1200 series quaternary pump and an Agilent Eclipse-XDB-C18 analytical column (150 mm × 4.6 mm, 5 μm) was applied to separate and detect the analytes. The aqueous mobile phase was initially composed of 40% methanol (component A) and 60% formic/format buffer at pH 2 (component B). While running the solvent gradient program, from 0 to 5 min the concentration of component A linearly increased to 80%, then ramped to 100% at 10 min (the total run takes 10 min). The mobile phase flow rate and injection volume were set at 1 mL min^{-1}, and 15 μL, respectively. DAD monitoring wavelengths were adjusted at 210 nm for KET, IBU, and DIC and 220 nm for NAP, respectively.

GC–MS analyses were made on an Agilent 7890 gas chromatograph (Centerville Road, Wilmington, NC, USA) equipped with a multi-mode inlet (MMI) of an electrospray ionization mode (ESI, 1200 eV) and a mass range of 50–700 amu. The separation was performed on a HP-5 fused silica capillary column (30 m × 0.32 mm id, 0.25 mm film thickness) provided by the Supelco (Bellefonte, PA, USA). Helium (purity 99.999%) was employed as the carrier gas at a flow rate of 1.0 mL min^{-1}. Samples were injected in programmed temperature vaporization (PTV) mode, so that within 5 min the injector temperature was raised to 280 °C. The GC oven was initially held at 70 °C for 2 min, then ramped to 280 °C at 25 °C min^{-1}, and finally held for 15 min. The solvent cut-off time was set at 5 min. The retention times for the studied compounds were as follows:

IBU (8.5 min), NAP (10.5 min), KET (11.9 min), and DIC (13.1 min).

Four ions with respect to each NSAID (as trimethylsylil (TMS) derivative) were selected under SIM acquisition mode (dwell time of 100 ms) for identification and quantification as follows: IBU-TMS, *m*/*z* 161, 234, 263, 278; KET-TMS, *m*/*z* 105, 282, 311, 326; DIC-TMS, *m*/*z* 214, 242, 277, 367; NAP-TMS, *m*/*z* 185, 243, 287, 302.

2.3. Sample procedure

For breaking down the drug–protein bindings and liberation of the drugs from the plasma matrix, a protein-precipitation treatment was performed as follows: 200 μL of TFA was added to 1.0 mL of plasma and the resulting mixture was vigorously vortexed for 2 min. The mixture was placed on ice for 5 min, kept for 2 min at room temperature, and then centrifuged at 4000 rpm for 5 min. The supernatant solution was transferred into a 5-mL sample vial and made up to the mark with ultrapure water while being pH-adjusted at 1.0. Finally, the mixture underwent the extraction procedure under the optimal conditions. Meanwhile, in order to reduce the matrix effects in the urine sample, the sample was diluted 2:5 with ultrapure water and the pH of the sample was adjusted to 1.0 as well.

In the extraction step, a 5-mL sample solution was poured into a sample vial. The mix of acetone (disperser solvent, 1.00 mL) and n-dodecane/TOPO (95:5, v/v) (extraction solvent, 200 μL), was quickly transferred into the solution using a 2.00 mL syringe. A turbid solution (water/acetone/*n*-dodecane with 5% TOPO) instantly appeared in the vial. Following that, the NSAIDs were extracted from the sample into the super tiny drops of the extraction solvent through a 5-min vortex agitation. The resulting mixture was then centrifuged for 5 min at 4000 rpm. After that, the dispersed tiny drops of the extraction solvent were collected on the top of the aqueous phase (160 ± 5 μL) and transferred into a 2 mL centrifuge tube. In the next step, 20 μL basic MeOH was swiftly added into the above tube and vortexed for 30 s. In this step, the analytes were extracted to the acceptor phase (basic MeOH). After the centrifugation, the lower acceptor phase was withdrawn, of which, 15 μL was injected into the HPLC-DAD. The rest of acceptor phase (5 μL) was vaporized to dryness and combined with 5 μL of MSTFA, of which, 2 μL was injected into the GC–MS. As mentioned earlier, the derivatization step was carried out in the injection port of GC–MS (in-port derivatization) (Danielson et al., 2000). The scheme for the proposed sample preparation method was presented in Fig. 1.

3. Results and discussion

3.1. Optimization of DLLME-related parameters

(a) Kind and volume of the extraction solvent

Initial experiments were carried out using 1.00 mL of acetone and 200 μL of an extraction solvent whilst no salt was used. For this purpose, a number of extraction solvents such as dichloromethane, 1-octanol, 1-undecanol, n-dodecane, n-dodecane/TOPO (95:5, v/v), and 1-octanol/TOPO (95:5, v/v) were evaluated to find the best extraction solvent. Also, it

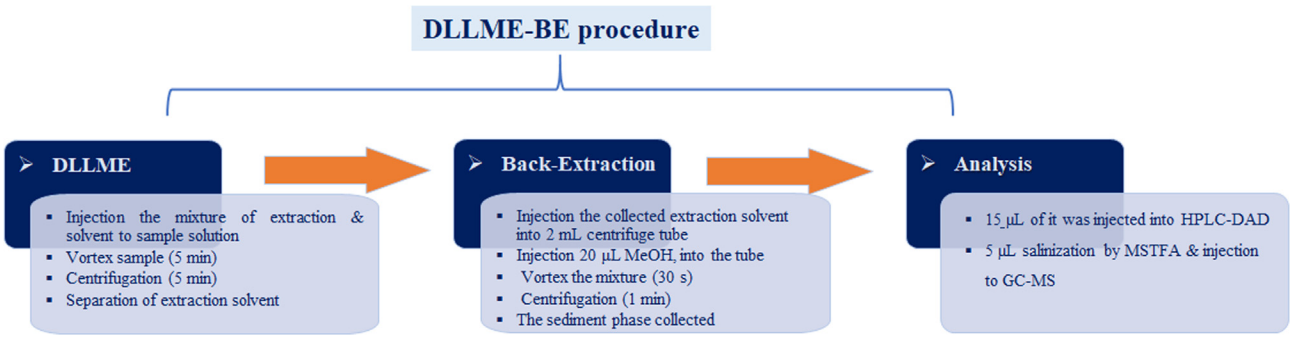

Fig. 1 A scheme for presentation of the introduced sample preparation method.

should be noted that the polarity of *n*-dodecane was increased by the inclusion of TOPO, which led to the enhancement of the extraction efficiency. As can be clearly deducted from Fig. 1S (Electronic supplementary materials (ESM)), amongst the solvents used, n-dodecane/TOPO (95:5, v/v) provided the highest extraction efficiencies. Thus, it was selected as the optimal extraction solvent for further experiments. Additionally, the impact of the content of TOPO in *n*-dodecane was evaluated in the span of 1–15% v/v. The results indicated that the transport of analytes increased with the rise of TOPO content from 1 to 5%. Further increases in the carrier content from 5 to 15% had almost negligible influence on the extraction efficiencies (data not shown).

In order to assess the influence of the extraction solvent volume, different volumes of the selected extraction solvent (75, 100, 150, 200, and 250 μL) and a fixed volume of acetone (1.00 mL) were employed Fig. 2A). It was found out that by increasing the volume from 75 to 200 μL, the extraction efficiencies were enhanced. Thus, 200 μL of n-dodecane/TOPO (95:5, v/v) was selected as the optimal solvent extraction volume. It is also noted that the volume of the extraction solvent collected was determined to be 160 ± 5 μL (n = 15).

(b) Kind and volume of disperser solvent

For assessing the impact of disperser solvent on the extraction efficiency of the method and obtaining the optimal disperser solvent, different organic solvents including ACN, MeOH, and acetone were investigated, each at 1.00 mL level. Amongst these, acetone exhibited better miscibility with *n*-dodecane and the aqueous solution and also delivered the highest extraction efficiencies. It should be noted that ACN and MeOH are immiscible with *n*-dodecane as well. Hence, acetone was selected as the disperser solvent in the following experiments. Further on, various volumes of acetone (0.25, 0.50, 1.00, 1.50, and 2.00 mL) in conjunction with the extraction solvent (200 μL) were tested. The results clearly indicated that extraction efficiencies were increased up to 1.00 mL and decreased thereafter, which could be attributed to the fact that the implication of high content of acetone leads to a higher solubility of NSAIDs in the aqueous phase. Consequently, 1.00 mL of acetone was selected as the optimum disperser solvent volume.

(c) Effect of pH

As a rule of thumb, pH is a major contributor involved in the extraction efficiency especially for acidic/basic analytes. With regard to pK_a of the analytes, the transport of the analytes from the sample solution to the extraction solvent is highly affected by the variation of pH in the sample solution. As the studied analytes are acidic, the alteration of pH should change their respective forms (i.e., neutral molecular and/or ion form). As expected, under low pH values the neutral forms of the analytes are predominantly present in the sample solution, so that they are easily extracted to the extraction solvent. As clearly shown in Fig. 2B, the extraction efficiencies dropped down with an increase in the pH of the donor phase from 1 to 3. Therefore, pH 1 was chosen for the subsequent experiments.

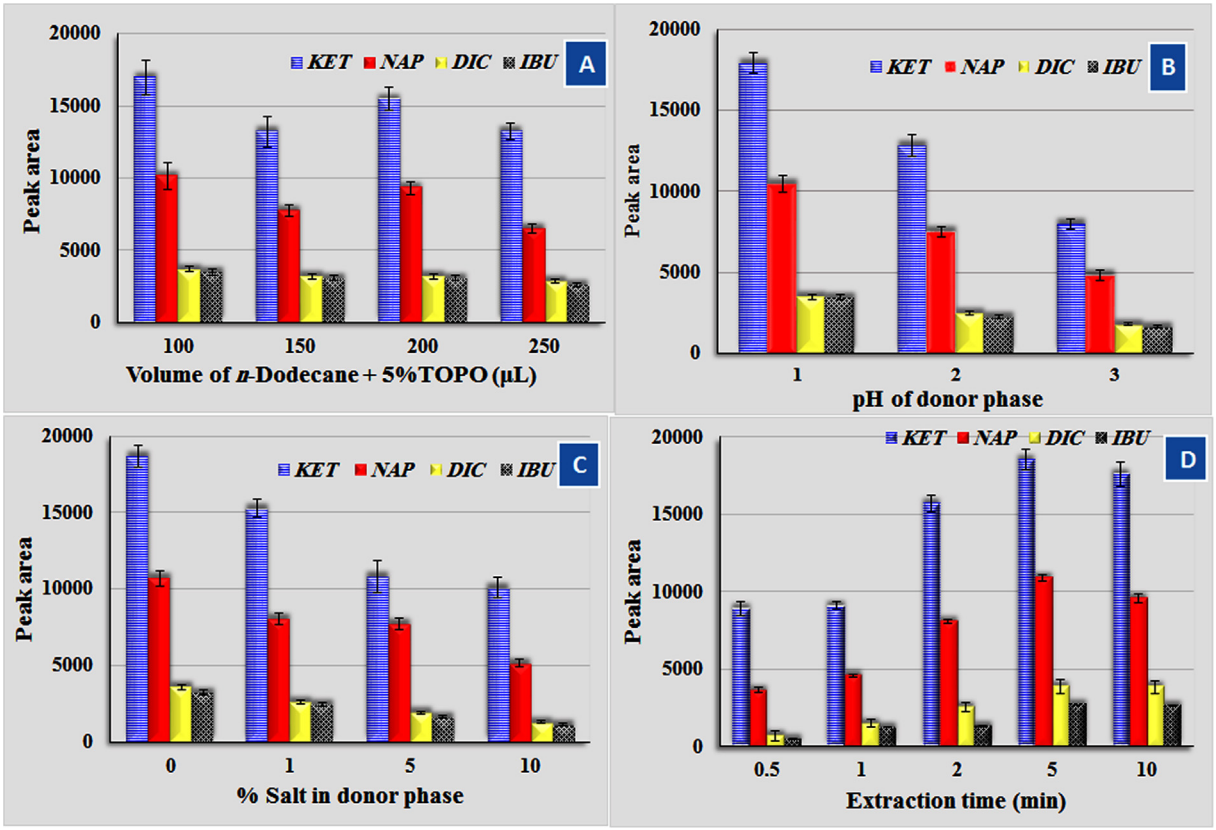

Fig. 2 Effect of volume of extraction solvent (A), pH of sample solution (B), salt addition (C) and extraction time (D). (Disperser solvent: 1 mL acetone; acceptor solvent: MeOH).

(d) Salt addition and extraction time

It was revealed that the extraction efficiencies fell down with increasing the salt concentration from 0 to 10.0% (w/v), as depicted in Fig. 2C. It was also observed that the appearance of the turbid solution was hampered when higher amounts of salt (≥10%) were added to the sample solution. This could be explained by the fact that the miscibility of acetone with water is reduced as a result. Hence, no salt was added in the subsequent experiments.

In a further study, the influence of extraction time was also examined in the span of 0.5–10 min whilst the other experimental conditions were set at their respective fixed levels. The results clearly exhibits that the extraction efficiency increased with extending the extraction time from 0.5 to 5 min. Beyond 5 min, the impact of extraction time on the extraction efficiency was found to be negligible Fig. 2D). Thus, 5 min was used as the best extraction time in further experiments.

3.2. Optimisation of back-extraction conditions

In order to find the best back-extraction solvent, a number of organic solvents such as MeOH, ACN, dihexylether (DHE), tetrahydrofuraan (THF) and water, which all are immiscible with n-dodecane/TOPO (95:5, v/v), were tested. They were all basified to facilitate the back-extraction of the analytes. It was observed that the mass transfer of the analytes from the extraction solvent to the basified back-extraction solvent was higher than the corresponding values to the aqueous acceptor. The results also demonstrated that the highest attainable extraction efficiencies were obtained when basic methanol was implemented as the acceptor phase (Supporting Information Fig. 2S (ESM)). We should say that the back-extraction stage in this work is performed to address two major issues as following:

1- In fact, we did extract the biological samples using the DLLME stage alone and what we achieved was the organic extract phase (2-dedcane and TOPO) full of unwanted materials which overwhelmed the whole phase resulting in an enhancement of background signals and interferences within the matrix.
2- Additionally, the introduction of the above-mentioned extract phase (2-dedcane and TOPO) to GC and HPLC could hamper the whole analysis and does severe damages to the used chromatographic instruments.

4. Method validation

The validation of the method was carried out under the optimal conditions by investigating the following parameters: linear range, preconcentration factor (PF), MDL and LOQ, precision (RSD %), accuracy (Error %) and matrix effect. The LOQs were calculated according to the following criterium:

Table 1 Figures of merit of the proposed sample preparation method.

Method	Matrix	NSAIDs	Calibration parameters		Figure of merits							
			LDR[a] (μg L^{-1})	R^2	*PF*	*MDL* (μg L^{-1})	*LOQ* (μg L^{-1})	*ER* %	% *RSD* (n = 3)			
									Intra-assay		Inter-assay	
									20 (μg L^{-1})	100 (μg L^{-1})	20 (μg L^{-1})	100 (μg L^{-1})
DLLME-BME/ HPLC-DAD	Plasma	*KET*	4.0–400.0	0.992	37	1.0	4.0	74.0	4.8	4.2	5.2	4.8
		NAP	4.0–400.0	0.981	23	1.0	4.0	46.0	3.5	3.1	4.1	3.6
		DIC	25–400.0	0.988	19	6.0	25.0	38.0	5.9	5.7	6.2	5.8
		IBU	25–400.0	0.981	22	6.0	25.0	44.0	5.3	3.9	5.7	4.5
	Urine	*KET*	2.0–400.0	0.998	78	0.5	2.0	78.0	5.7	5.2	6.3	6.2
		NAP	2.0–400.0	0.972	47	0.5	2.0	47.0	6.1	5.5	6.7	6.2
		DIC	15–400.0	0.992	36	5.0	15.0	36.0	5.2	4.7	5.9	5.0
		IBU	15–400.0	0.986	47	5.0	15.0	47.0	7.8	7.0	8.1	7.5
	Water	*KET*	0.5–400.0	0.999	215	0.1	0.5	86.0	3.5	3.1	4.2	3.5
		NAP	1.0–400.0	0.996	145	0.3	1.0	58.0	3.2	3.9	5.0	3.7
		DIC	5.0–400.0	0.999	97	1.0	5.0	38.8	4.6	2.8	5.1	4.4
		IBU	5.0–400.0	0.982	129	1.0	5.0	51.6	4.9	4.5	5.2	4.9
DLLME-BME/ GC–MS	Plasma	*KET*	2.5–400.0	0.982	38	0.5	2.5	76.0	11.7	10.2	12.1	11.3
		NAP	2.0–400.0	0.999	24	0.5	2.0	48.0	13.5	12.3	13.0	12.5
		DIC	5.0–400.0	0.999	21	1.0	5.0	42.0	10.6	9.9	11.1	10.8
		IBU	5.0–400.0	0.998	23	1.0	5.0	46.0	12.5	11.0	13.0	11.7
	Urine	*KET*	1.0–400.0	0.999	75	0.3	1.0	75.0	14.0	9.8	14.2	10.1
		NAP	1.0–400.0	0.989	49	0.3	1.0	49.0	3.5	2.7	4.7	3.9
		DIC	2.0–400.0	0.999	37	0.5	2.0	37.0	11.6	10.5	11.7	11.3
		IBU	2.0–400.0	0.989	48	0.5	2.0	48.0	12.9	11.4	12.5	12.0
	Water	*KET*	0.5–400.0	0.997	219	0.1	0.5	82.0	14.5	14.1	14.2	13.8
		NAP	0.5–400.0	0.998	149	0.1	0.5	56.0	12.3	11.1	12.6	11.5
		DIC	0.5–200.0	0.999	101	0.2	0.5	39.6	14.2	13.4	14.5	14.1
		IBU	0.5–200.0	0.968	135	0.2	0.5	54.0	13.8	12.5	13.5	13.2

[a] Linear dynamic range.

The lowest detectable concentration level at which the accuracy and precision fall within the ranges of 80–120 and 0–20%, respectively.

Matrix effect was also evaluated in drug-free water, urine, and plasma samples, according to the FDA recommendations (Food and Drug Administration, 2013). As presented in Table 1, the calibration curves were linear over the range of 0.5–400.0 μg L^{-1} for DLLME-BE followed by HPLC-DAD and GC–MS. The MDLs were calculated on the basis of signal-to-noise ratios of 3, which were in the ranges of 0.1–1.0 for GC–MS and 0.1–6.0 μg L^{-1} for HPLC-DAD. The LOQs obtained were in the range of 0.5–5.0 and 0.5–25 μg L^{-1} for GC–MS and HPLC-DAD, respectively. Inter- and intra-assay (as the index of precision/repeatability) were calculated with three determinations at two concentration levels (20 and 100 μg L^{-1} for each drug (QCs)). The PFs were determined to be in the range of 19–219. According to the FDA guidelines, matrix effect is the direct or indirect alteration or interference in a response due to the presence of unintended analytes or other interfering substances in the sample. No significant interfering peaks were observed at the retention times of the target analytes following the extraction from the water and real samples. In this study, the matrix effect was determined by comparing the absolute peak areas in the neat solutions with those obtained at each level of the standard fortified (50 and 200 μg L^{-1} (QCs)) for the six real samples (Matuszewski et al., 2003). Additionally, all validation steps for the DLLME-BE/GC–MS procedure were carried out and the results are presented in Table 2.

4.1. Analysis of real samples

The applicability of the proposed method for the measurement of the studied analytes in two real biological fluid samples (urine and plasma) was assessed. The samples were collected from two patients who had already consumed the drugs and then placed in the fridge until the time of analysis.

Then, the target analytes were extracted under the optimal conditions. The Error, and RSD% for the analysis of NAP, KET, DIC and IBU in plasma and urine samples were determined based on three measurements Table 2. Relative recoveries (RR%) were calculated using the following expression:

$$RR\% = 100 \times (C_{found}C_{real})/C_{added}$$

where C_{found} and C_{added} are the concentrations of the analytes in the samples after and before the addition of a known quantity of the standard solutions. Fig. 3 represents the HPLC-DAD chromatograms of the real urine (A) and plasma (B)

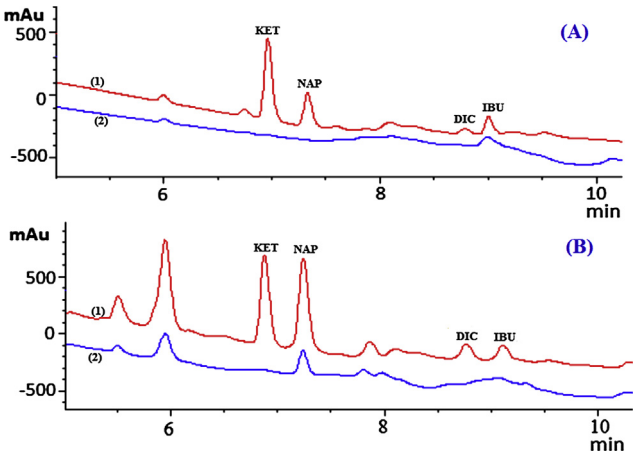

Fig. 3 HPLC-DAD chromatograms of a real urine 1 (A) and plasma 2 (B) samples before (2) and after (1) spiking with NSAIDs standard solution at concentration level of 200 μg L^{-1}.

Table 2 Analytical results for the extraction and determination of NSAIDs in urine and plasma samples.

Sample	Analyte	Found ± SD[b] (n = 3) C_{added}[a] = 0 μg L^{-1}	Found ± SD (n = 3) C_{added} = 200 μg L^{-1}	% RR[d]	Matrix effect% (n = 6)	
					50 μg L^{-1}	200 μg L^{-1}
Urine 1	*KET*	< *LOD*[c]	195.3 ± 9.5	97.6	62.4	65.3
	NAP	< LOD	204.3 ± 10.2	102.2	65.3	70.2
	DIC	< LOD	210.4 ± 9.8	105.2	38.7	39.0
	IBU	246.5 ± 11.5	451.2 ± 23.2	93.4	28.3	29.5
Plasma 1	*KET*	< LOD	185.4 ± 9.6	92.7	90.7	95.2
	NAP	< LOD	191.3 ± 10.2	95.7	98.6	97.4
	DIC	< LOD	204.7 ± 7.9	102.4	104.3	100.7
	IBU	170.7 ± 5.7	365.4 ± 16.4	97.4	75.6	77.9
Urine 2	*KET*	< LOD	198.4 ± 8.7	99.2	62.4	65.3
	NAP	56.4 ± 20.7	230.0 ± 11.5	86.8	65.3	70.2
	DIC	< LOD	201.4 ± 9.5	100.7	38.7	39.0
	IBU	< LOD	188.2 ± 8.9	94.1	28.3	29.5
Plasma 2	*KET*	< LOD	201.4 ± 10.5	102.1	90.7	95.2
	NAP	42.9 ± 2.5	225.8 ± 9.9	91.5	98.6	97.4
	DIC	< LOD	194.4 ± 8.9	97.2	104.3	100.7
	IBU	< LOD	205.7 ± 10.6	102.9	75.6	77.9

[a] Added concentration.
[b] Standard deviation.
[c] Not detected.
[d] Relative recovery.

Please cite this article in press as: Ghambarian, M. et al., An efficient sample preparation method based on dispersive liquid–liquid microextraction associated with back extraction for trace determination of acidic pharmaceuticals. Arabian Journal of Chemistry (2018), https://doi.org/10.1016/j.arabjc.2018.02.010

sample before (2) and after (1) fortified with the NSAIDs standard solution at 200 μg L^{-1}, affirming the existence of IBU (A) and NAP (B). In all real samples analyzed, the presence of NSAIDs was verified by both GC–MS analysis and the comparison of the obtained mass spectrum Fig. 4-1B and 4-2B) with the MS database of instrument library Fig. 4-1A and 4-2A. Fig. 4C and D depict the GC–MS chromatograms of the non-fortified real plasma and urine sample after the extraction process, respectively. Fig. 4-1B and 4-2B presents the mass spectra (SIM mode) of detected NSAIDs in the urine and plasma samples. As can be observed, the GC–MS results reaffirm the presence of IBU and NAP in the urine and plasma samples.

4.2. Comparative study

The efficiency of the presented method in terms of extraction time, initial volume of biological fluid samples, LOD, LOQ, % ER and intra-day precision was compared with the others reported in the literature and summarized in Table 3. Briefly, the main advantages of the proposed method are rapidness, simplicity and requirement to less amount of organic solvent

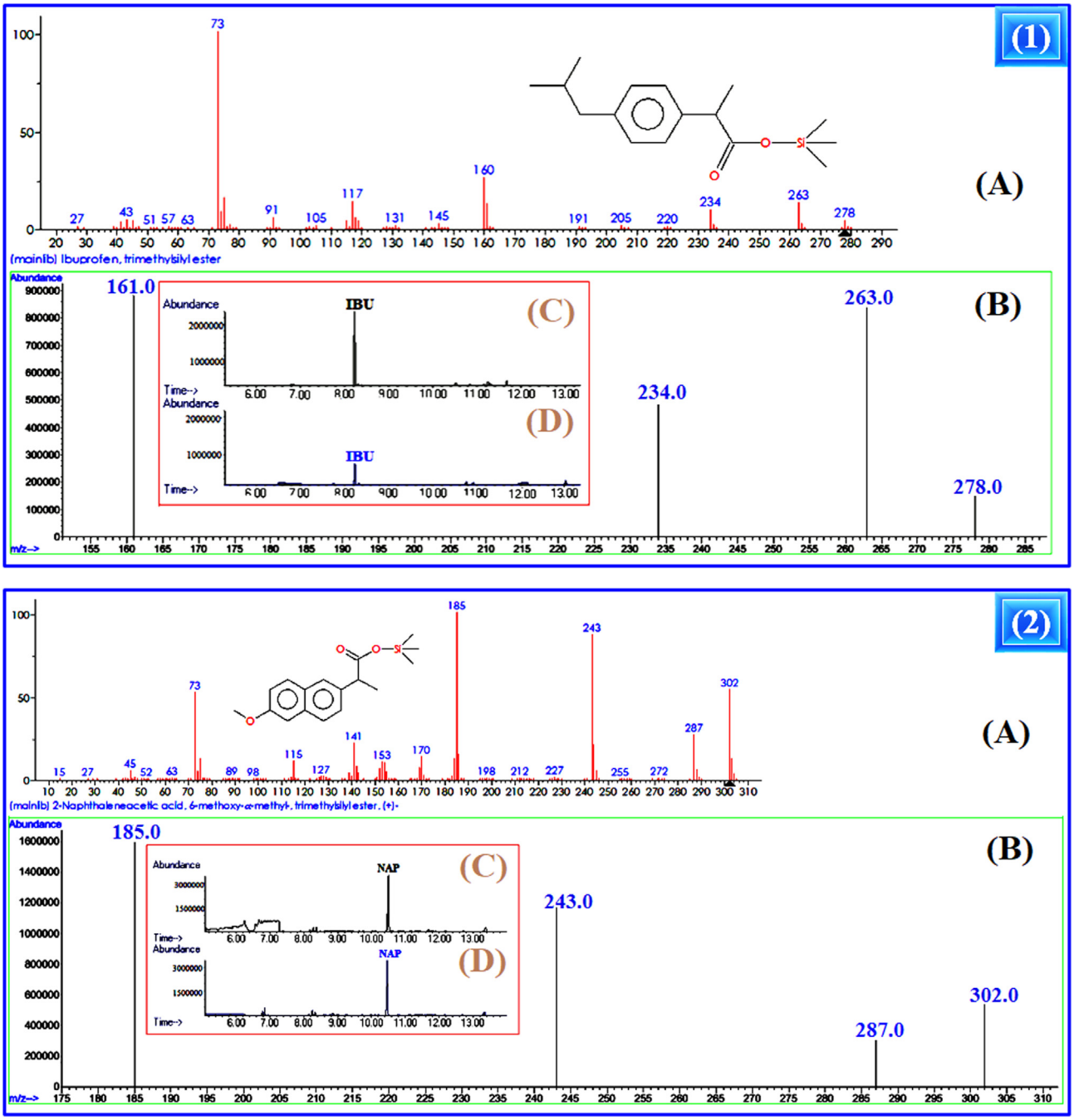

Fig. 4 MS spectrum in database of instrument library for IBU-TMS (1A) and NAP-TMS (2A) in comparison with MS spectrum for IBU-TMS (1B) and NAP-TMS (2B). The chromatograms B and C in Scheme 1 and 2 show the GC–MS chromatograms of the non-spiked real plasma and urine samples after extraction, respectively.

Table 3 Comparison of the proposed method with other methods applied for the extraction and determination of NSAIDs.

Sample	Extraction method	Determination	Extraction time (min)	Sample volume (mL)	ER%	RSD%	MDL (ng mL^{-1})	LOQ (ng mL^{-1})	Ref
Urine	USE-AALLME[a]	GC-FID	4.5	5.0	69.0–80.0	4.3–4.8	0.1–1.0	0.4–5.0	Barfi et al. (2015)
Waste water	HF-LPME[b]	GC–MS	300	50.0	–	–	7.1–58.0	23.6–297.4	Larsson et al. (2014)
Pharmaceuticals	HF-LPME	HPLC-DAD/FLD	150	50.0	–	–	1.9–52.9	6.3–176.6	Ramos et al. (2009)
Urine, plasma	MEPS[c]	HPLC-PDA	300	5.0	52.0–80.0	< 7.3	0.03	0.1	Locatelli et al. (2014)
Urine	DLLME-SFO[d]	HPLC-UV	–	5.0	95.7–115.6	–	3.4–5.2	10	Shukri et al. (2015)
Water, plasma, urine	DLLME-BE	HPLC-DAD	10.5	Plasma: 1.0 Urine:	36.0–86.0	2.8–8.1	0.1–6.0	0.5–25.0	This work
Water, plasma, urine		GC–MS		2.0 Water: 5.0	37.0–82.0	2.7–14.5	0.1–1.0	0.5–5.0	

[a] Ultrasound-enhanced air assisted liquid–liquid microextraction.
[b] Hollow fiber–liquid phase microextraction.
[c] Microextraction by packed sorbent.
[d] Dispersive liquid–liquid microextraction based on solidification of floating organic drople.

and real sample for the analysis. Since low consumption of immiscible organic solvent is applied in the back-extraction step, this method presented low LODs and LOQs compared to those of DLLME-SFO method (Ramos and Payán, 2009; Locatelli et al., 2014; Barfi et al., 2015; Larsson et al., 2014; Shukri et al., 2015). Meanwhile, the results obtained indicate that DLLME in tandem with back-extraction is a promising joint method for the analysis of NSAIDs at low concentration levels in biological fluid samples and can be applied for the analysis of a wide variety of different polar compounds.

5. Concluding remarks

Within this study, a simple and efficient method for the extraction and determination of selected NSAIDs in biological fluid samples was developed. The method was based on the combination of DLLME with back-extraction technique in which two immiscible organic solvents were utilized. We should say that the back-extraction stage in this work is performed to address two major issues as following:

1- In fact, we did extract the biological samples using the DLLME stage alone and what we achieved was the organic extract phase (2-dedcane and TOPO) full of unwanted materials which overwhelmed the whole phase resulting in an enhancement of background signals and interferences within the matrix.
2- Additionally, the introduction of the above-mentioned extract phase (2-dedcane and TOPO) to GC and HPLC could hamper the whole analysis and does severe damages to the used chromatographic instruments.

In the present method, a further step (back extraction of targets) is required and therefore the time needed for present method is higher than other dispersive liquid-liquid microextraction methods.

The extraction procedure is relatively simple, efficient, quick, and low-cost compared to other methods. Other advantages of this joint method include the compatibility with GC due to using organic acceptor solvent, minimization of the matrix effect, application of the selective derivatization of polar purified compounds by MSTFA prior to GC analysis and provision of a high clean-up in the analysis of highly complicated biological fluid matrices.

Finally, this simple and robust DLLME-BE method could be implemented in medical/other laboratories for monitoring NSAIDs in human fluids.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.arabjc.2018.02.010.

References

Zhang, H., Du, Z., Me, M., 2013. Simultaneous trace determination of acidic non-steroidal anti-inflammatory drugs in purified water, tap water, juice, soda and energy drink by hollow fiber-based liquid-phase microextraction and ultra-high pressure liquid chromatography coupled to tandem mass spectrometry. Talanta 109, 177–184.

Kim, S., Aga, D.S., 2007. Potential ecological and human health impacts of antibiotics and antibiotic-resistant bacteria from wastewater treatment plants. J. Toxicol. Environ. Health B 10, 559–573.

Bazregar, M., Rajabi, M., Yamini, Y., Asghari, A., Hemmati, M., 2016. Tandem air-agitated liquid–liquid microextraction as an efficient method for determination of acidic drugs in complicated matrices. Anal. Chim. Acta 917, 44–52.

Barfi, B., Asghari, A., Rajabi, M., Goochani Moghadam, A., Mirkhani, N., Ahmadi, F., 2015. Comparison of ultrasound-enhanced air-assisted liquid–liquid microextraction and low-density solvent-based dispersive liquid–liquid microextraction methods for determination of nonsteroidal anti-inflammatory drugs in human urine samples. J. Pharm. Biomed. Anal. 111, 297–305.

ParrillaVázquez, M., ParrillaVázquez, P., Martínez Galera, M., Gil García, M., Uclés, A., 2013. Ultrasound-assisted ionic liquid dispersive liquid–liquid microextraction coupled with liquid chromatography-quadrupole-linear ion trap-mass spectrometry for simultaneous analysis of pharmaceuticals in wastewaters. J. Chromatogr. A 1291, 19–26.

Toledo-Neira, C., Álvarez-Lueje, A., 2015. Ionic liquids for improving the extraction of NSAIDs in water samples using dispersive liquid–liquid microextraction by high performance liquid chromatography-diode array–fluorescence detection. Talanta 134, 619–626.

Rezaee, M., Assadi, Y., Millani, M.R., Aghaee, E., Ahmadi, F., Berijani, S.J., 2006. Determination of organic compounds in water using dispersive liquid–liquid microextraction. J. Chromatogr. A 1116, 1–9.

Guo, L., Lee, H.K., 2013. Vortex-assisted micro-solid-phase extraction followed by low-density solvent based dispersive liquid–liquid microextraction for the fast and efficient determination of phthalate esters in river water samples. J. Chromatogr. A 1300, 24–30.

Simão, V., Merib, J., Dias, A.N., Carasek, E., 2016. Novel analytical procedure using a combination of hollow fiber supported liquid membrane and dispersive liquid–liquid microextraction for the determination of aflatoxins in soybean juice by high performance liquid chromatography – Fluorescence detector. Food. Chem. 196, 292–300.

Ghambarian, M., Tajabadi, F., Yamini, Y., Esrafili, A., 2016. Dispersive liquid–liquid microextraction with back extraction using an immiscible organic solvent for determination of benzodiazepines in water, urine, and plasma samples. RSC adv. 6, 114198–114207.

Mzukisi Madikizela, L., Tawanda Tavengwa, N., Chimuka, L., 2018. Applications of molecularly imprinted polymers for solid-phase extraction of non-steroidal anti-inflammatory drugs and analgesics from environmental waters and biological samples. J. Pharm. Biomed. Anal. 147, 624–633.

Kamaruzaman, S., Marsin Sanagi, M., Endud, S., Aini Wan Ibrahim, W., Yahaya, N., 2013. MCM-41 solid phase membrane tip extraction combined with liquid chromatography for the determination of non-steroidal anti-inflammatory drugs in human urine. J. Chromatogr. B 940, 59–65.

Sultan, M., Stecher, G., Stöggl, W.M., Bakry, R., Zaborski, P., Huck, C.W., El Kousy, N.M., Bonn, G.K., 2005. Sample pretreatment and determination of non-steroidal anti-inflammatory drugs (NSAIDs) in pharmaceutical formulations and biological samples (blood, plasma, erythrocytes) by HPLC-UV-MS and micro-HPLC. Curr. Med Chem. 12 (5), 573–588.

Gouda, A.A., Kotb El-Sayed, M.I., Amin, A.S., El Sheikh, R., 2013. Spectrophotometric and spectrofluorometric methods for the determination of non-steroidal anti-inflammatory drugs: a review. Arab. J. Chem. 6, 145–163.

Rodriguez, I., Quintana, J.B., Carpinteiro, J., Carro, A.M., Lorenzo, R.A., Cela, R., 2003. Determination of acidic drugs in sewage water by gas chromatography–mass spectrometry as tert.-butyldimethylsilyl derivatives. J. Chromatogr. A 985, 265–274.

Yu, Z., Peldszus, S., Huck, P.M., 2007. Optimizing gas chromatographic–mass spectrometric analysis of selected pharmaceuticals and endocrine-disrupting substances in water using factorial experimental design. J. Chromatogr. A 1148, 65–77.

Rice, S.L., Mitra, S., 2007. Microwave-assisted solvent extraction of solid matrices and subsequent detection of pharmaceuticals and personal care products (PPCPs) using gas chromatography–mass spectrometry. Anal. Chim. Acta 589, 125–132.

Danielson, N.D., Gallagher, P.A., Bao, J.J., 2000. Chemical reagents and derivatization procedures in drug analysis. Encyclopedia Anal. Chem., 7042–7076

Food and Drug Administration, U. S. Department of Health and Human Services, 2013.

Matuszewski, B.K., Constanzer, M.L., Chavez-Eng, C.M., 2003. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal. Chim. Acta 75, 3019–3030.

Ramos Payán, M., Bello López, M.A., Fernández-Torres, R., Pérez Bernal, J.L., Callejón Mochón, M., 2009. HPLC determination of ibuprofen, diclofenac and salicylic acid using hollow fiber-based liquid phase microextraction (HF-LPME). Anal. Chim. Acta 653, 184–190.

Locatelli, M., Ferrone, V., Cifelli, R., Carmine Barbacane, R., Carlucci, G., 2014. Microextraction by packed sorbent and high performance liquid chromatography determination of seven non-steroidal anti-inflammatory drugs in human plasma and urine. J. Chromatogr. A 1367, 1–8.

Barfi, B., Asghar, A., Rajabi, M., Goochani Moghadam, A., Mirkhani, N., Ahmadi, F., 2015. Comparison of ultrasound-enhanced air-assisted liquid–liquid microextraction and low-density solvent-based dispersive liquid–liquid microextraction methods for determination of nonsteroidal anti-inflammatory drugs in human urine samples. J. Pharm. Biomed. Anal. 111, 297–305.

Larsson, E., al-Hamimi, S., ÅkeJönsson, J., 2014. Behaviour of nonsteroidal anti-inflammatory drugs and eight of their metabolites during wastewater treatment studied by hollow fibre liquid phase microextraction and liquid chromatography mass spectrometry. Sci. Total. Environ. 485–6, 300–308.

Shukri, D.S.M.D., Marsin Sanagi, M., Wan Ibrahim, W.A., Zainal Abidin, N.N., Aboul-Enein, H.Y., 2015. Liquid chromatographic determination of NSAIDs in urine after dispersive liquid-liquid microextraction based on solidification of floating organic droplets. Chromatographia 78, 987–994.

Please cite this article in press as: Ghambarian, M. et al., An efficient sample preparation method based on dispersive liquid–liquid microextraction associated with back extraction for trace determination of acidic pharmaceuticals. Arabian Journal of Chemistry (2018), https://doi.org/10.1016/j.arabjc.2018.02.010